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## CLAIMS

- 1. Method which allows cells to acquire the capacity to produce a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises the following steps:
- a) transforming said cells by at least one introduction of a missense mutation in a target codon of a gene encoding a protein required for the growth of said cells, said protein synthesized from the gene thus mutated no longer being functional;
- b) where appropriate, culturing the cells obtained in step a) in a culture medium containing a nutrient compensating for the loss of functionality of said protein thus mutated; and
- c) culturing the cells obtained in step a) or b) in a culture medium containing the amino acid encoded by said target codon.
- 2. Method according to claim 1, characterized in that the culture medium of step c) does not contain the nutrient required by the loss of functionality of said mutated protein.
  - 3. Method according to either of claims 1 and 2, characterized in that step c) for culturing said cells comprises a series of cultures of said cells in a culture medium containing the amino acid encoded by said target codon, each of said cultures of the series being prepared as far as obtaining the stationary growth phase and followed by washing of the cells obtained, the number of cultures of the series being sufficient to allow the selection of mutations which increase the suppression of said missense mutation of said mutated gene.
- 35 4. Method according to one of claims 1 to 3, characterized in that the missense mutation is chosen from missense mutations which spontaneously reverse

only at very low frequency, of the order of organism from at least 1015.

- Method according to one of claims 1 characterized in that the missense mutation transforms a target codon of a gene encoding a protein required for the growth of said cell, into /a codon which, comparison with the target codon, exhibits a change of at least two bases, preferably three bases.
- Method according to one of claims 1 5, characterized in that the target/codon encodes an amino acid which has a small steric volume.
  - Method according to one of claims characterized in that the /target codon encodes amphiphilic amino acid.
- Method according to one of claims 7. 15 1 to characterized in that the/target codon encodes an amino acid which has a steric volume smaller substantially equal to/the steric volume of the amino acid encoded by the missense mutation.
- 20 9. Method according to one of claims 5 to 8, characterized the in /that target codon cysteine.
  - 10. Method according to one of claims characterized in/that the amino acid encoded by the missense mutation is valine or isoleucine.
  - Method / according to one of claims 1 to 10, characterized / in that step a) for transforming said cells is carried out using a vector comprising a sequence of/said gene encoding a protein required for the growth of said cells, including said missense mutation.
  - Method according to claim 11, characterized in 12. that said vector is a plasmid vector.
  - Method for selecting cells capable of producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises steps a), where appropriate b), and c) of a method according to one of claims 1 to 12, and selecting the cells/capable of growing in step c).

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14. Method for selecting cells according to claim 13, characterized in that it also comprises a step d) for culturing the cells in step c) in a culture medium containing said amino acid encoded by said target codon, the concentration of said amino acid possibly being at a concentration higher than the concentration of said amino acid used in step c), and choosing the cells sensitive to the concentration of said amino acid used in step d).

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15. Method for selecting cells according to either of claims 13 and 14 characterized in that the aminoacyl-tRNA synthetase which recognizes the amino acid encoded by said missense mutation of said selected cells is capable of charging onto one of its associated tRNAs an unconventional amino acid or an amino acid other than said amino acid encoded by said missense mutation.

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16. Method for selecting cells according to claim 15, characterized in that the nucleic acid sequence of the gene encoding said aminoacyl-tRNA synthetase includes at least one mutation compared with the sequence of the corresponding wild-type gene.

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17. Method for selecting cells according to claim 16, characterized in that said mutation has not been introduced by a technique of genetic recombination.

18. Cell obtained using a method according to one of claims 1 to 17.

19. Isolated cell capable of producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises an aminoacyl-tRNA synthetase which recognizes a given amino acid and which is capable of charging onto one of its associated tRNAs an unconventional amino acid or an amino acid other than said given amino acid, and in that the nucleic acid sequence of the gene encoding said aminoacyl-tRNA synthetase includes at least one mutation compared with the sequence of the corresponding wild-type gene, said mutation not having

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been introduced a technique by of genetic recombination.

Cell according to claim 18 or 19, characterized in that it is a prokaryotic or eukaryotic cell.

Cell according to claim 20, characterized in that it is a prokaryotic cell.

- Cell according to one of claims 18 to 21, characterized in that it is chosen from the following cells deposited at the CNCM (Collection Nationale de Culture de Microorganismes [National Collection of Microorganism Cultures], Paris, France):
- E. coli strain deposited of the CNCM under the No. I-2025 on May 25, 1998,
- E. coli strain deposited at the CNCM under the b) No. I-2026 on May 25, 1998,
- E. coli strain deposited at the CNCM under the C) No. I-2027 on May 25, 1998,
- E. coli strain deposited at the CNCM under d) No. I-2339 on October 26, 1999,
- E. coli strain deposited at the CNCM under the 20 e) No. I-2340 on October 26, 1999, and
  - coli strain deposited at the CNCM under the No. I-2341 on Oqtober 26, 1999.
- Use of a method according to one of claims 1 to 23. for producing/protein the amino acid sequence of which comprises at least one unconventional amino acid.
  - 24. Use of a cell according to one of claims 18 to 22 for producing protein the amino acid sequence of which comprises at least one unconventional amino acid.
- 25. Process for producing a protein the amino acid 30 sequence of which comprises at least one unconventional amino acid / characterized in that it comprises the following steps:
- a) where appropriate, selecting a cell by a method 35 according to one of claims 13 to 17;
  - b) cultufing said cell selected in step a) or a cell according to one of claims 18 to 22 in a culture med um and under culture conditions which allow the gr $\phi$ wth of said cell; and

- said protein comprising at c) isolating least one acid unconvent ional amino from the supernatant and/or from the cell pellet obtained in step by.
- Process according to claim 25, characterized in that said culture medium of step b) which allows the growth of said cell contains said unconventional amino acid or a precursor thereof.
- Process according to claim 25, characterized in 27. that said unconventional amino acid is synthesized by said cell.
- Process according to claim 27, characterized in 28. that the synthesis of said unconventional amino acid is increased by genetic modification of said cell.
- Process according to one of claims 25 to 28, 29. characterized in that said cell is auxotrophic for the amino acid encoded by said target codon.
- Process according to one of claims 25 to 29, characterized in that said cell comprises a homologous or heterologous gene of interest the coding sequence of which includes at least one target codon.
- 31. Process according to claim 30, characterized in that step b) comprises the compounds required for inducing the synthesis of the protein encoded by said gene of interest.
- according to claim 32. Process 30 characterized in that the biological activity of the protein encoded by said gene of interest is at least partially conserved /after the incorporation of unconventional amin acid at the level of the target codon of said gene/of interest.
- Process according to one of claims 25 to 32, characterized in that the unconventional amino acid is chosen from unconventional amino acids of formula I of configuration L

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in which:

 $R_1$  or  $R_2$  /represents radicals containing a functional group capable of reacting selectively.

**(l)** 

34. Process according to claim 33, characterized in that the functional group is chosen from aldehyde, ketone, ethenyl, ethynyl and nitrile groups.

Process according to one of claims 25 to 34, 35. for protein functionalization.

Protein purification process, characterized in that it comprises the following steps:

incorporating into the amino acid sequence of said protein an unconventional amino acid containing a functional group /capable of reacting selectively, using a process /according to one of claims 25 to 35;

- bringing the solution containing the b) obtained in step a) into contact with a support comprising a compound capable of reacting specifically/with said functional group and of attaching specifically said protein; and
- isolating said protein attached to the support. c)
- 37. Process/ for attaching a protein to a chemical biochemica compound, characterized in 25 that comprises the following steps:
  - incorporating into the amino acid sequence of said protein/ by a process according to one of claims 25 to \$5, an unconventional amino acid containing functional group capable of reacting selectively;
  - bringing the protein obtained in step b) contact with said chemical or biochemical compound comprising a group capable of reacting

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specifically with said functional group medium allowing the reaction.

- Process according to claim 37, characterized in that said chemical or biochemical compound is, itself, attached to a solid support or is a constituent compound of a solid support.
- Process according to claim 37 for preparing a protein complex.
- Process according to claim 39, characterized in attached protein oż the chemical that the biochemical compound is chosen From therapeutic, cosmetic or diagnostic compounds.
- according claim 39 40. Process to characterized in that /the chemical or biochemical compound is chosen from compounds capable of modifying the biological activity of the attached protein.
- Process according to claim 39 that the chemical characterized in or biochemical chosen from compounds the biological compound is activity of which can be modified by the attached protein.
- 43. Process according to one of claims 39 to 42, characterized in that the chemical or biochemical compound is/chosen from compounds comprising a protein, a polynucleotide, a fatty acid, a sugar or a natural or synthetic/polymer.
- Protein obtained using a process according to one of  $\not$  laims 25 to 36.
- Protein according to claim 44, characterized in 45. that it is a recombinant protein.
- complex obtained using 46. Protein a process according to one of plaims 39 to 43.
- Use of a protein according to claim 44 or 45, or of a protein complex according to claim 46, as a diagnostic reagent.
- 48. Diagnostic process, characterized in that uses a protein according to claim 44 or 45, or a protein complex according to claim 46.

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